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Quantitative Structure-Activity Relationships for Organophosphate Enzyme Inhibition (Briefing Charts)

22 September 11

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14. ABSTRACT Organophosphates (OPs) are a group of pesticides that inhibit enzymes such as acetylcholinesterase. Numerous OP structural variants exist and toxicity data can be difficult to quickly obtain. To address this concern, quantitative structure-activity relationship (QSAR) models were developed to predict acetylcholinesterase, butyrycholinesterase, trypsin and chymotrypsin inhibition, key components in biologically-based dose-response (BBDR) models. The acetylcholinesterase database consisted of 747 structures developed from 69 peer reviewed publications. AMPAC and CODESSA descriptors (SemiChem, Inc.) were calculated for each compound. The acetylcholinesterase results show that the average nucleophilic reactive index for a carbon atom contributed most significantly to binding. A training R_2 of 0.73±0.01 and an external test set Q_2 of 0.62±0.06 was achieved. The QSAR models discussed in this seminar will complement OP BBDR modeling by filling critical data gaps for key parameter values, leading to better risk assessment and prioritization of animal and human toxicity studies especially for OPs lacking experimental data.			
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   b) Organophosphate structure and mechanism of toxicity
   c) Linking QSAR and OP PBPK/PD
2. Methods
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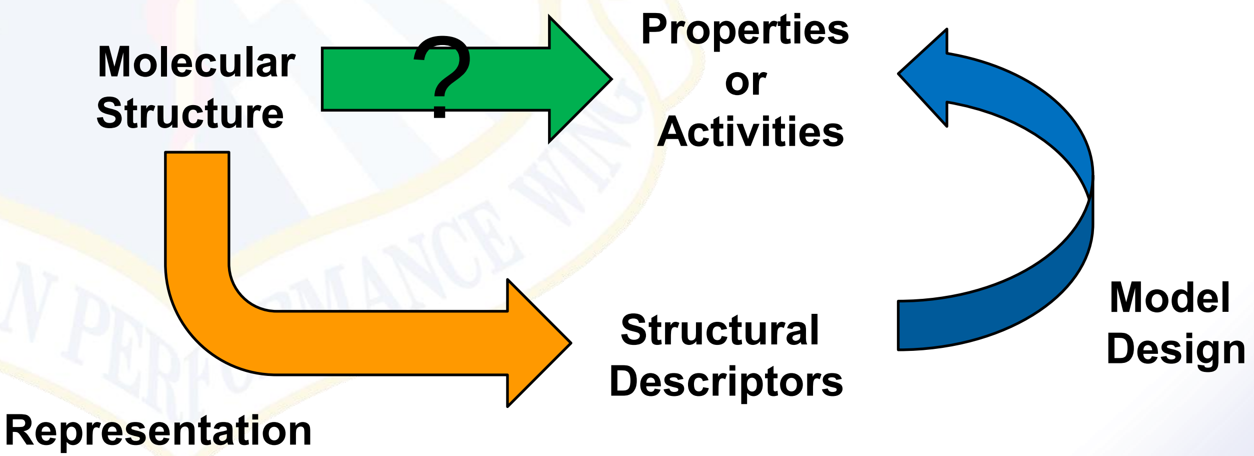
   a) Bimolecular rate constants
      i. Acetylcholinesterase & Butyrylcholinesterase
      ii. Trypsin & Chymotrypsin
4. Discussion

What is QSAR?

Quantitative Structure-Activity Relationship

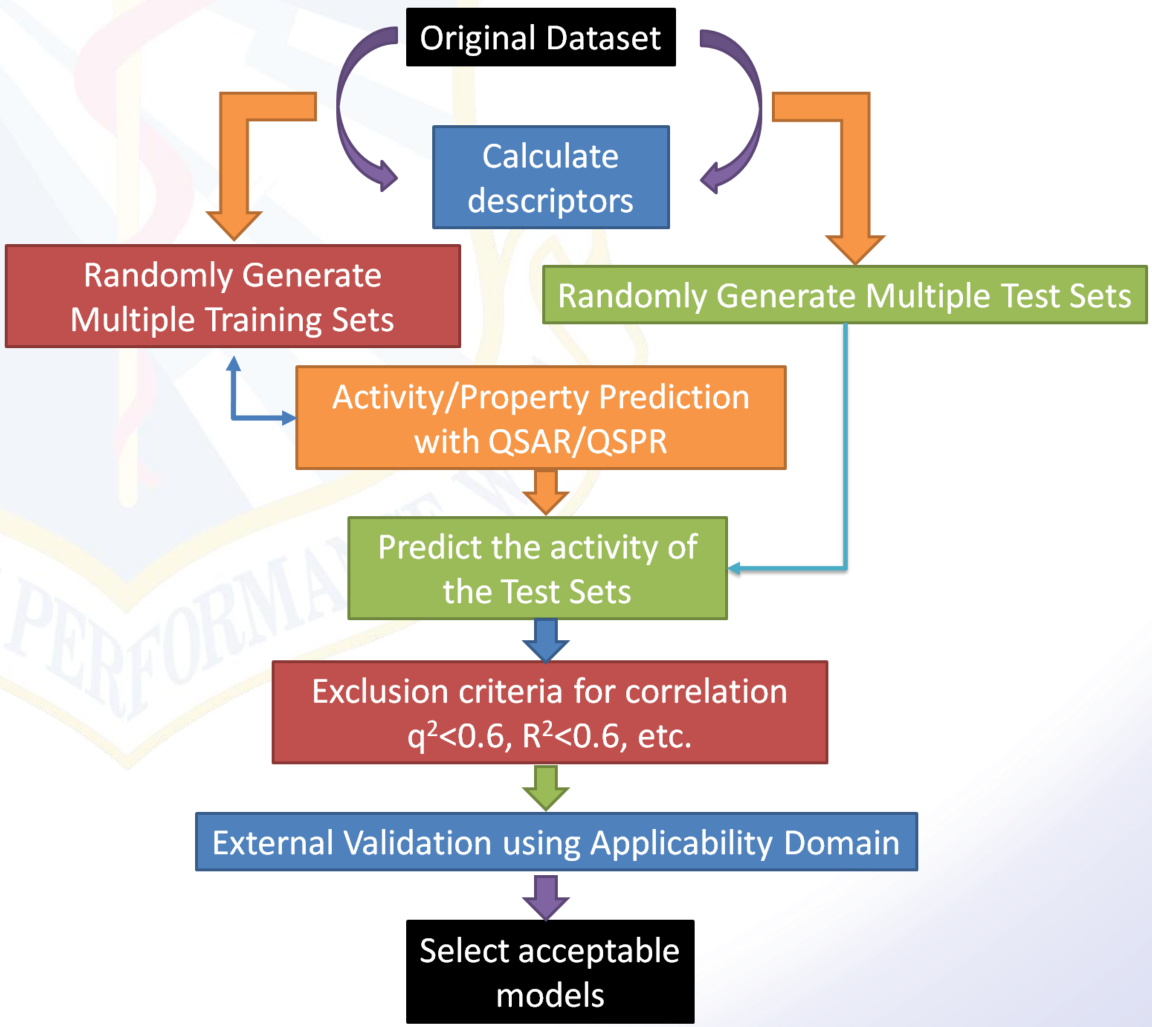
A technique used to quantify differences between biological activity and that of a molecular structure



There are guidelines/rules to this approach

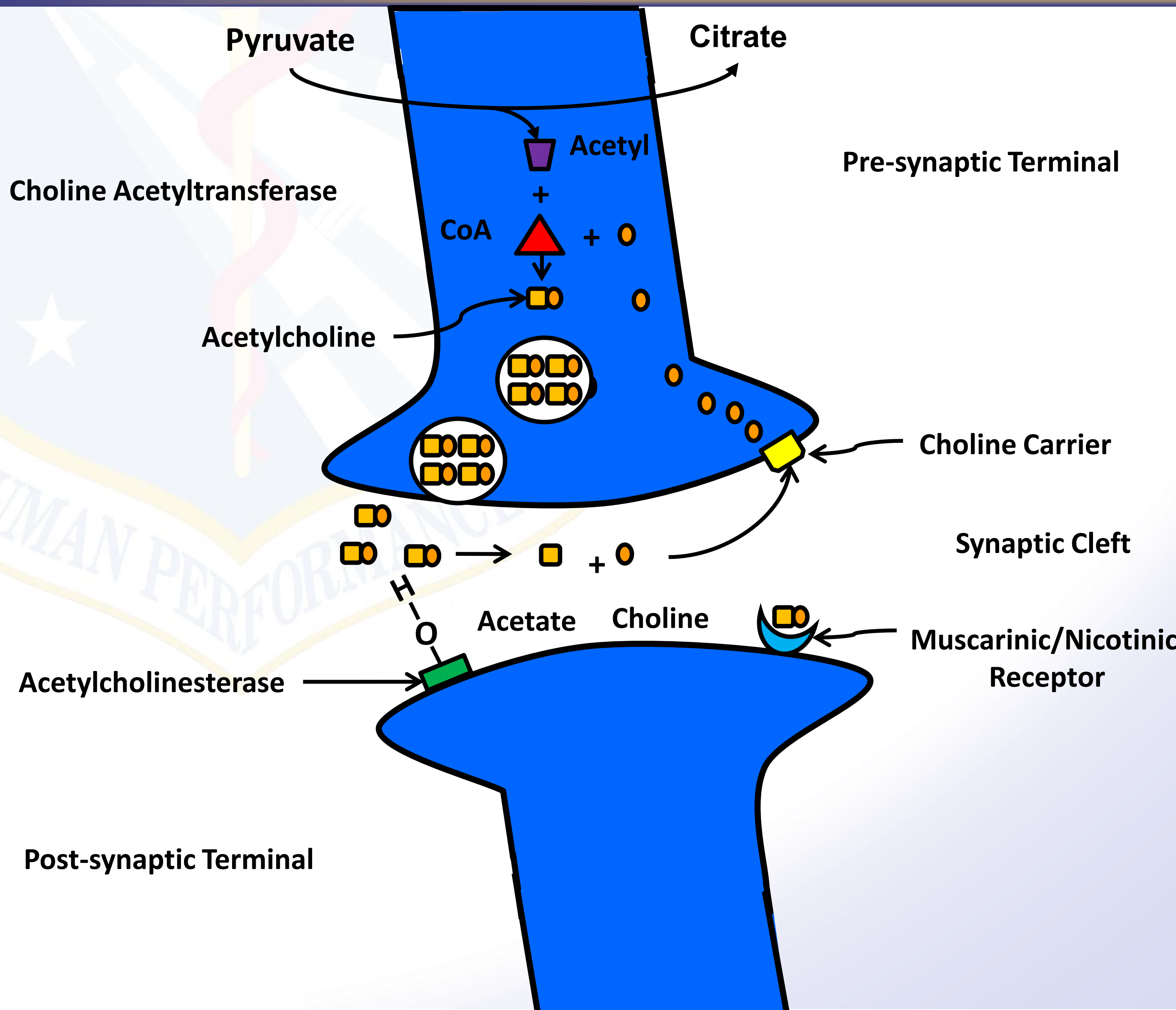
1. Choose well-defined Activity endpoints
2. Choose plausible molecular descriptors
3. Explore the data with statistics
4. Test hypotheses with new data (ie. iterate)

QSAR Overview



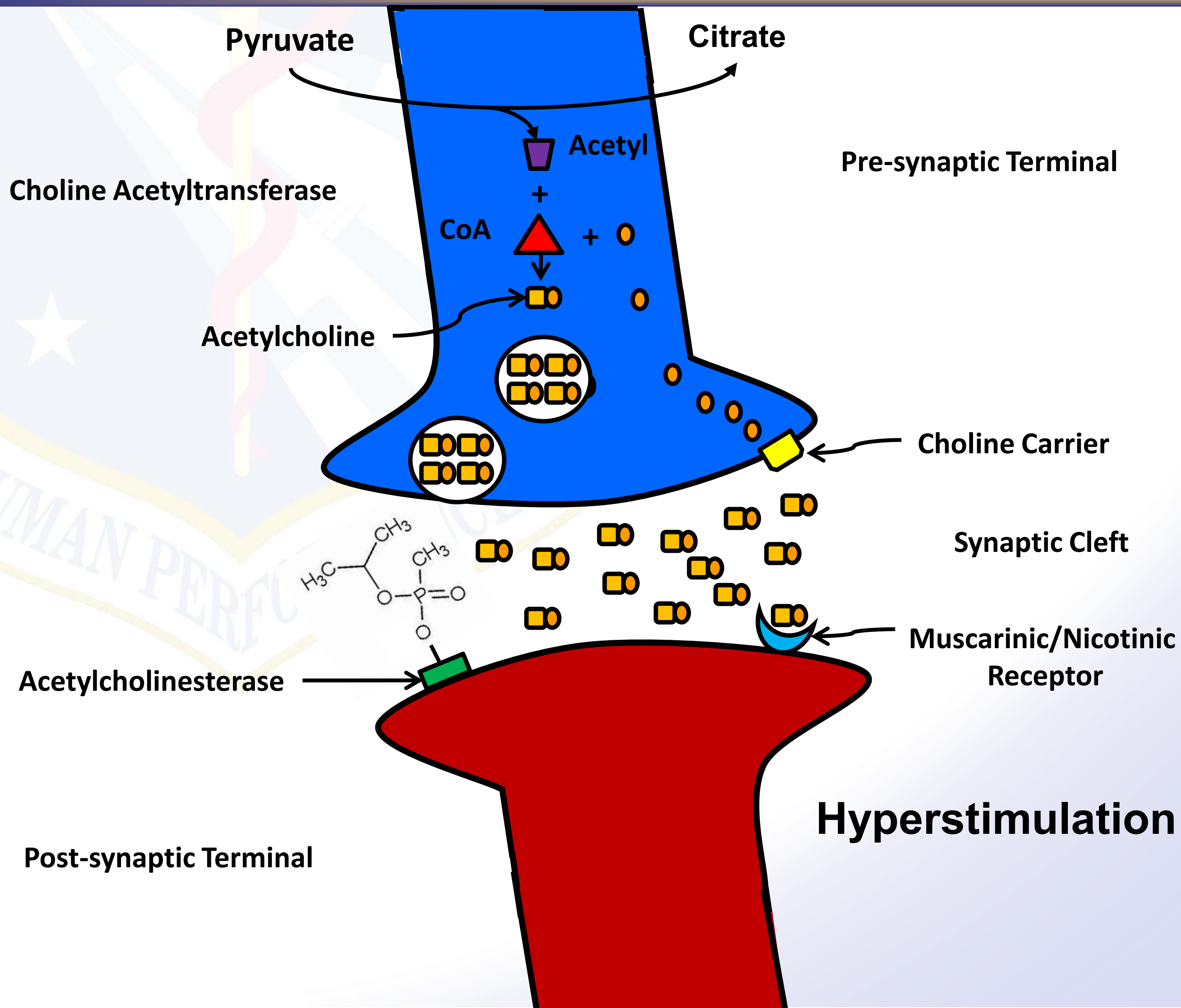
Cholinergic Nervous System

“Normal Mechanism of Action”

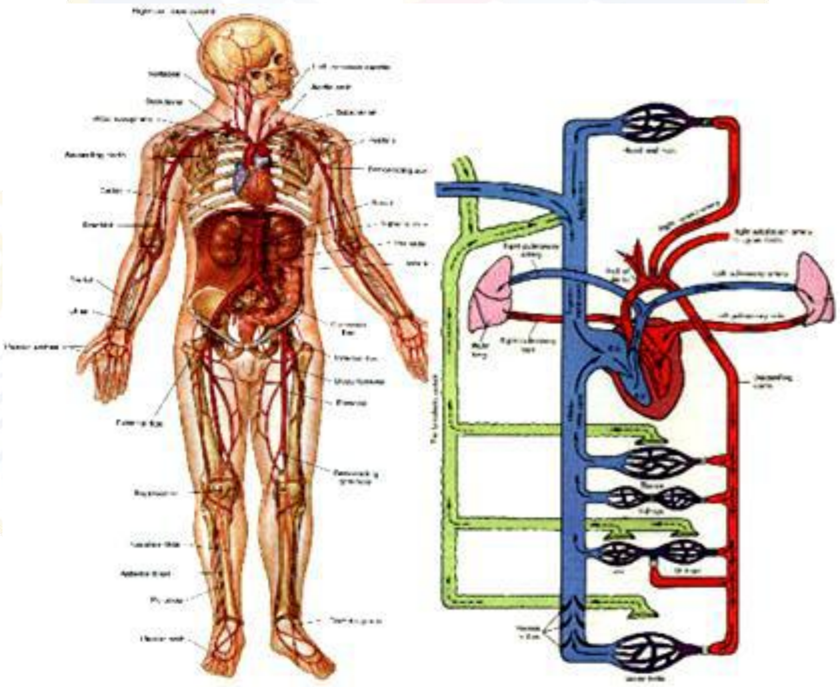


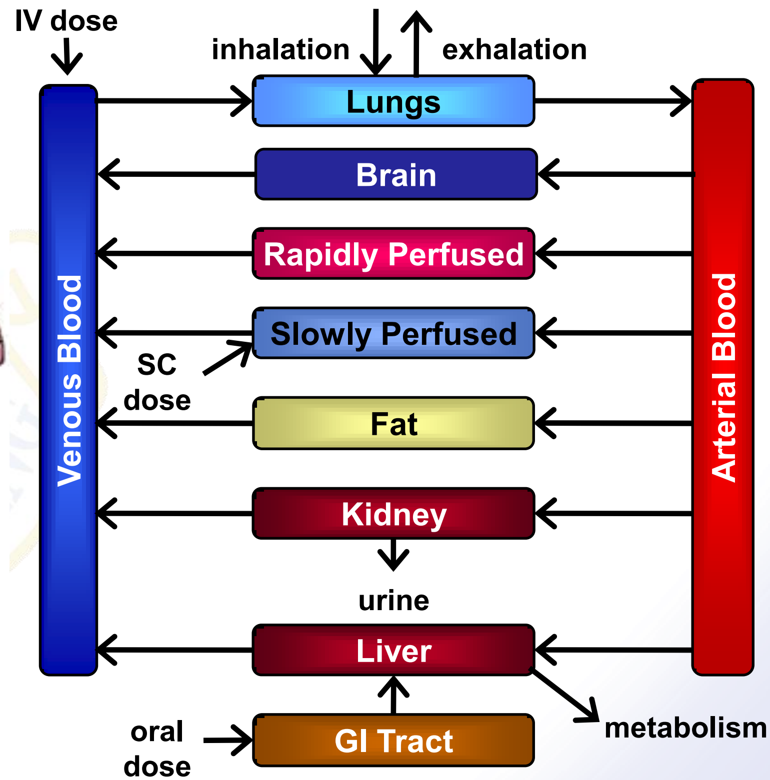
Cholinergic Nervous System

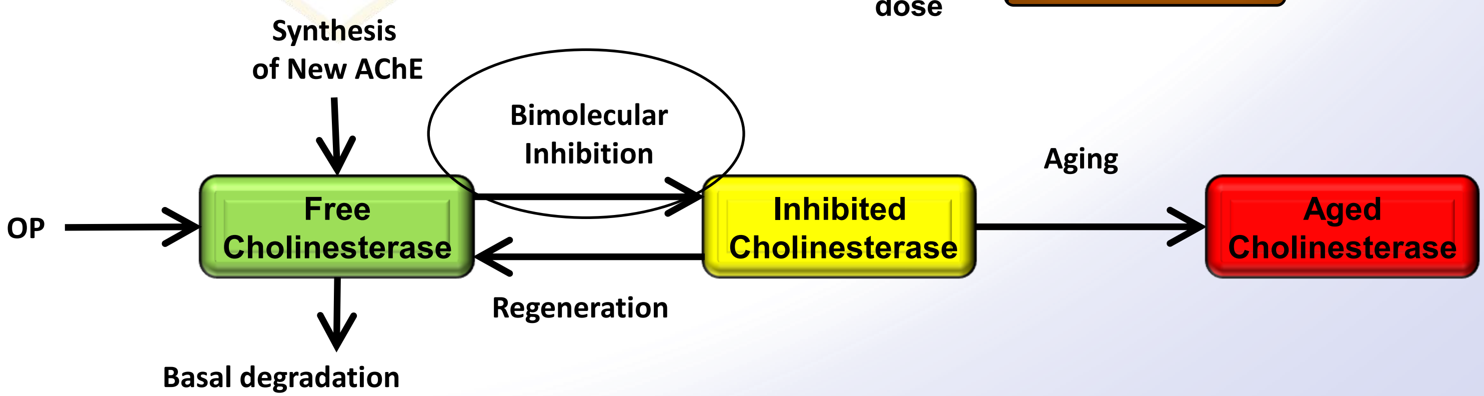
“OP Mechanism of Action”



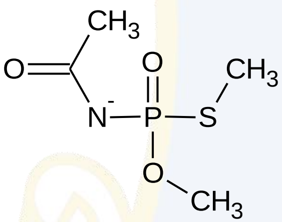
Physiologically Based Pharmacokinetic/Pharmacodynamic Modeling



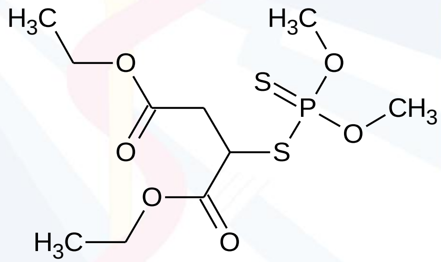




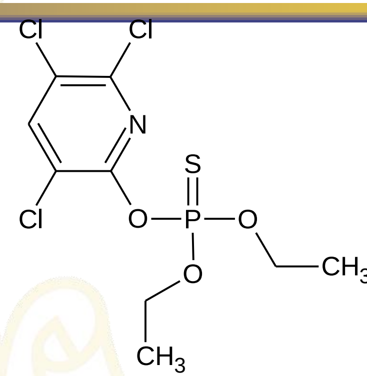
Organophosphate Structures



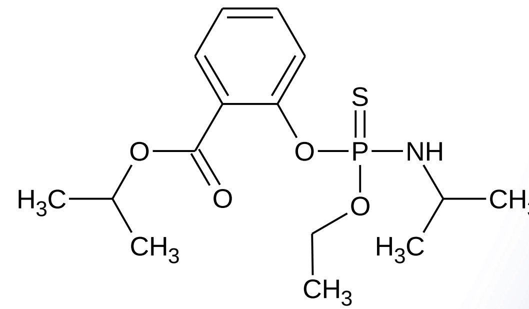
Acephate



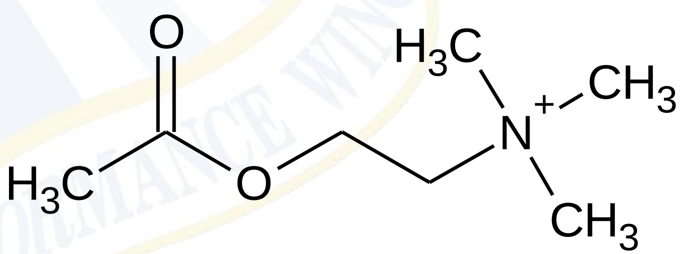
Malathion



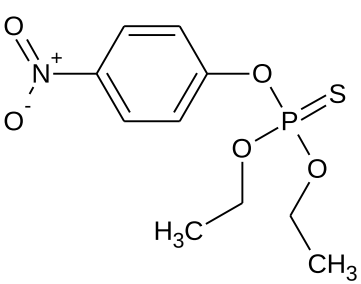
Chlorpyrifos



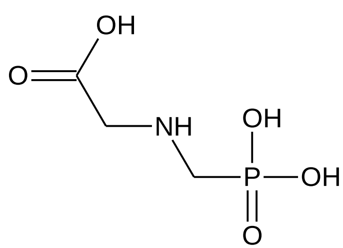
Isofenphos



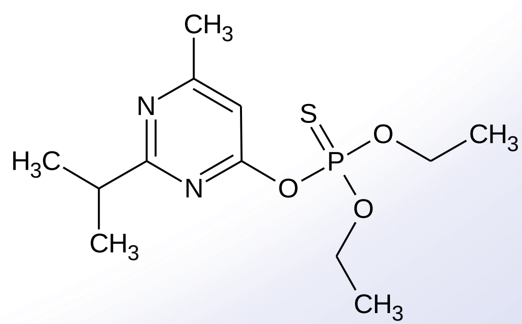
Acetylcholine



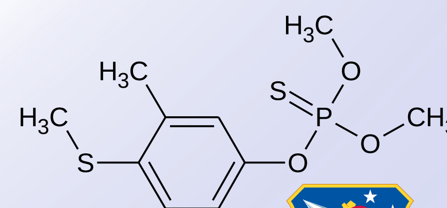
Parathion



Glyphosate

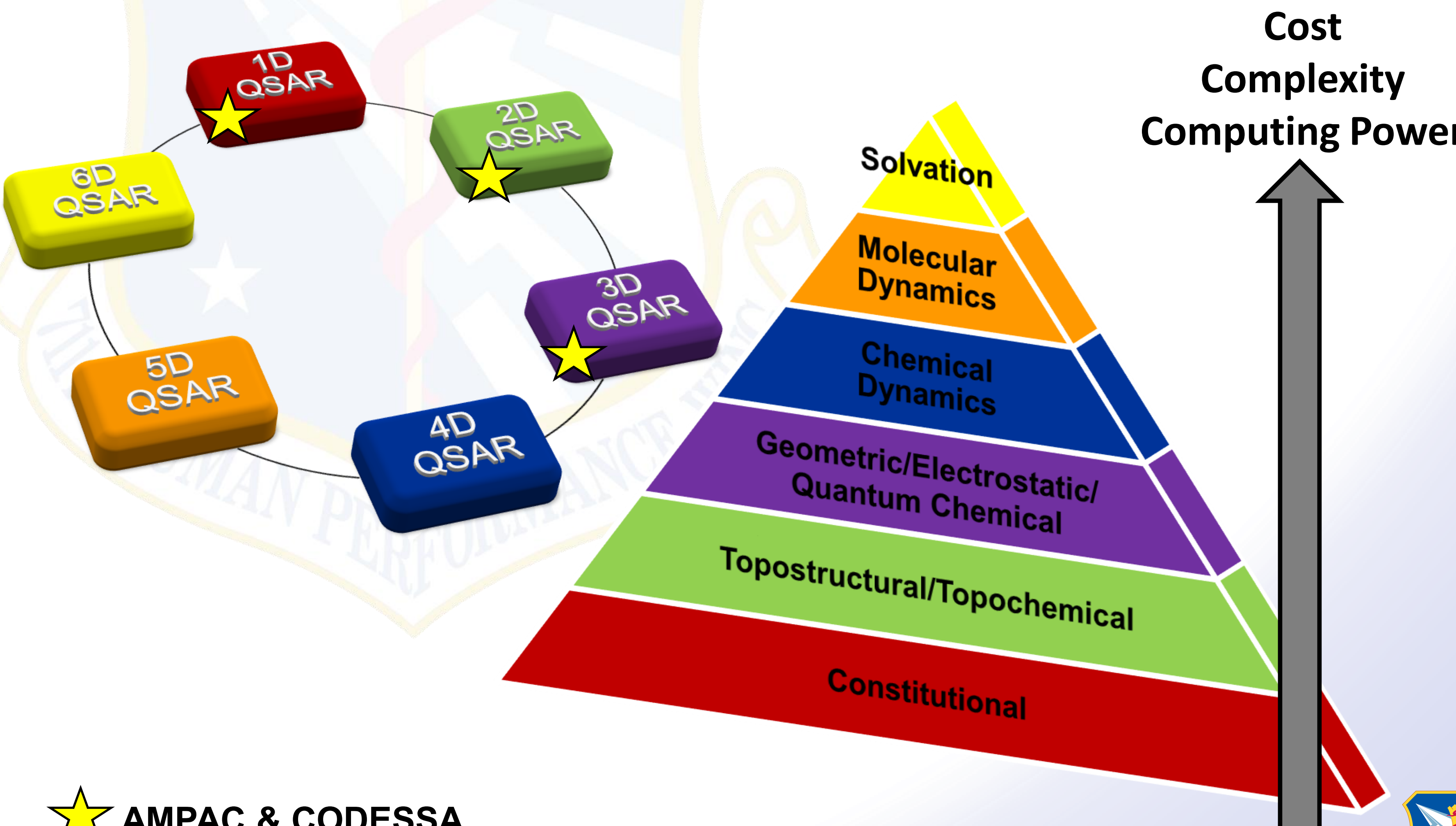


Diazinon



Fenthion

Physiochemical Descriptors



★ AMPAC & CODESSA

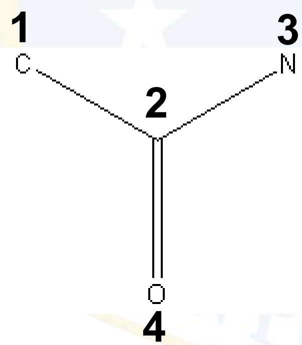
Constitutional Descriptors

Reflect molecular composition of compound without using geometry or electronic structure of molecule:

- **Number of atoms**
  - **Absolute and relative numbers of C, H, O, S, N, F, Cl, Br, I, P atoms**
- **Number of bonds**
  - **Absolute and relative numbers of single, double, triple and aromatic bonds**
- **Number of rings**
  - **Number of rings divided by the number of atoms, number of benzene rings, number of benzene rings divided by the number of atoms**
- **Molecular and average atomic weight**

Topostructural Descriptors

A molecular graph is made up of Edges and Vertices



(G)

Adjacency matrix (A)

$$
A\,(G)= \begin{array}{c} \\ (1) \\ (2) \\ (3) \\ (4) \end{array}
\begin{array}{c}
\begin{array}{cccc} (1) & (2) & (3) & (4) \end{array} \\
\left[\begin{array}{cccc} 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 1 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{array}\right]
\end{array}
$$

Distance matrix (D)

$$
D\,(G)= \begin{array}{c} \\ (1) \\ (2) \\ (3) \\ (4) \end{array}
\begin{array}{c}
\begin{array}{cccc} (1) & (2) & (3) & (4) \end{array} \\
\left[\begin{array}{cccc} 0 & 1 & 2 & 2 \\ 1 & 0 & 1 & 1 \\ 2 & 1 & 0 & 2 \\ 2 & 1 & 2 & 0 \end{array}\right]
\end{array}
$$

Many topostructural indices can be derived from matrices A and D

Regression Techniques

Linear Regression Examples

- Heuristic
- Partial Least Squares (PLS)
- Principle Component Regression (PCR)
- Orthogonal Projection to Latent Structures (OPLS)
- Ridge Regression

Non-Linear Regression Examples

- Support Vector Machines (SVM)
- Neural Networks (NN)
- Kernel Orthogonal Projection to Latent Structures (KOPLS)
- Kernel Partial Least Squares (KPLS)

Clustering Regression Examples

- k-nearest neighbor
- Random Forest

Regression Techniques

Overview of orthogonal projection to latent structures (O-PLS)

Original data set

PLS

- Harder to interpret
- More PLS components
- Orthogonal variation in X

Orthogonal data set

OPLS

- Evaluate orthogonal variation in principal components
- Identify source of orthogonal variation

OPLS treated data

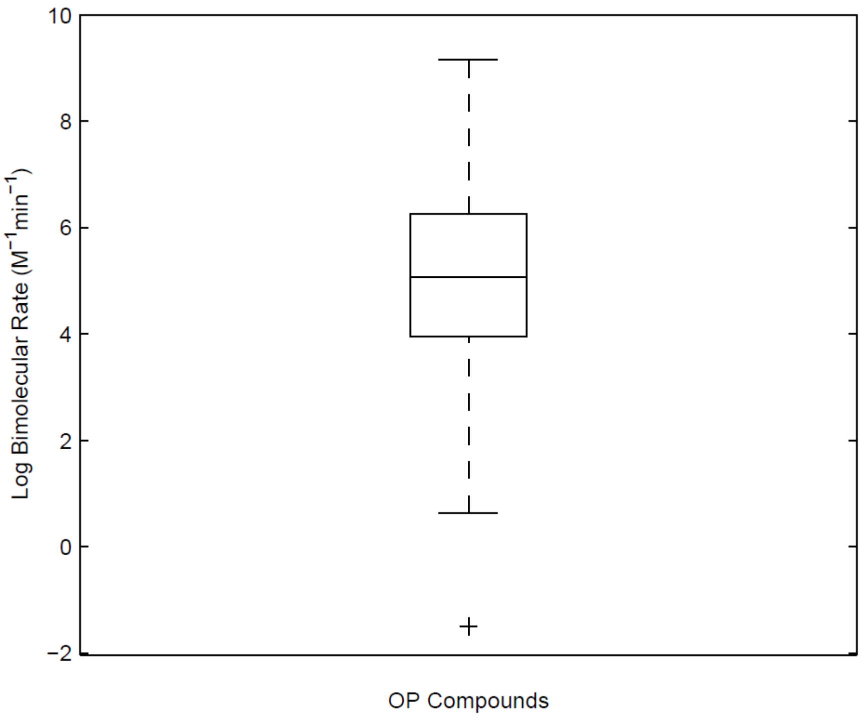
PLS

- Easier to interpret
- Fewer components
- More relevant

Adapted from Trygg and Wold 2002

Acetylcholinesterase Bimolecular Rate Constants ($M^{-1}min^{-1}$)

OP Library



Data collected from 69 peer-reviewed journal articles.

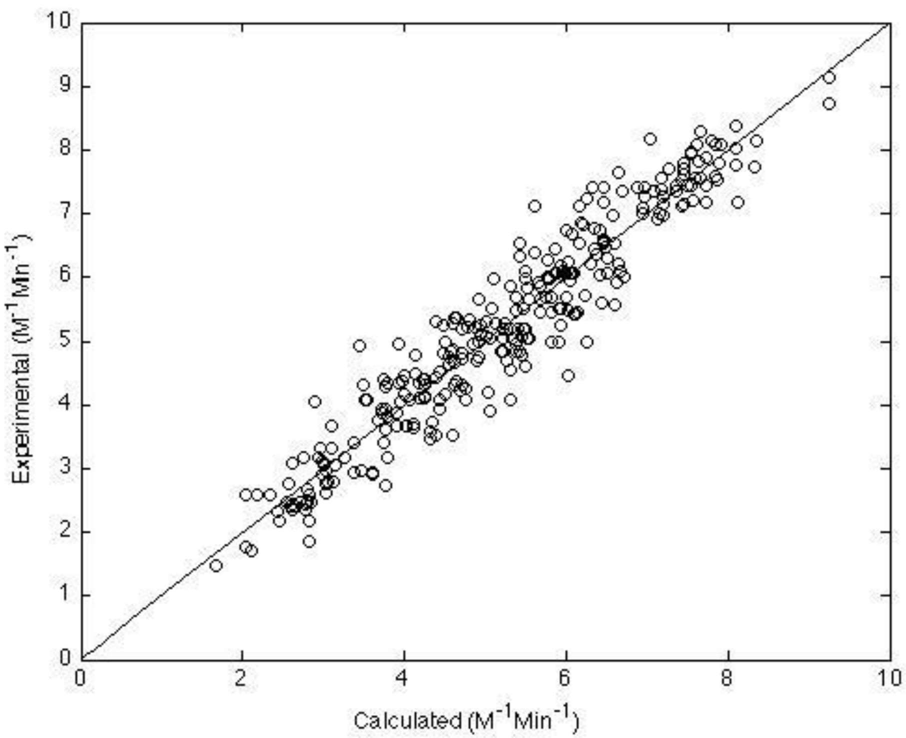
1068 observations

Table 1. Percentage of database that represents a particular temperature and species.

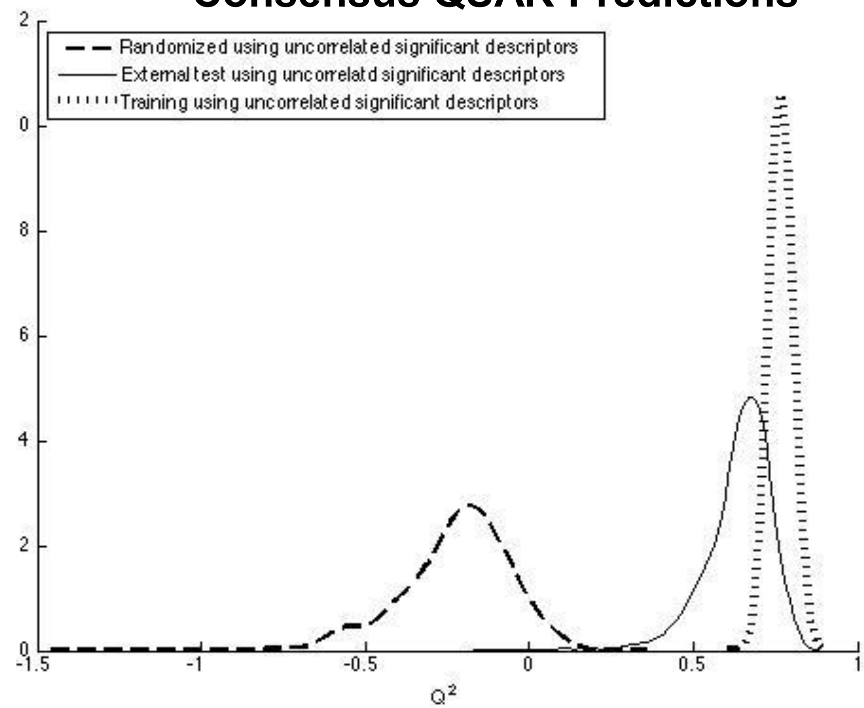
Species	Temperature (°C) 25	37	Unknown	5	30	22	27	Total Percent
Human	28.46	6.93	8.15	0.00	0.00	0.00	0.19	43.72
Bovine	21.44	3.56	0.09	0.09	1.22	0.00	0.00	26.40
Unknown	7.77	0.28	1.50	0.00	0.00	0.00	0.00	9.55
Fly	2.25	0.56	4.87	0.00	1.03	0.00	0.00	8.71
Rat	0.00	1.22	0.47	0.66	0.00	0.47	0.00	2.81
Hen	0.56	0.47	0.00	0.00	0.00	0.47	0.00	1.50
Rabbit	0.47	0.00	0.00	0.00	1.03	0.00	0.00	1.50
Eel	0.75	0.75	0.00	0.00	0.00	0.00	0.00	1.50
Cricket	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.66
Guinea Pig	0.19	0.00	0.00	0.00	0.00	0.47	0.00	0.66
Pig	0.00	0.66	0.00	0.00	0.00	0.00	0.00	0.66
Mouse	0.19	0.00	0.00	0.00	0.09	0.28	0.00	0.56
NHP	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.47
Catfish	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.47
Frog	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.47
Minipig	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.37
Total Percent	62.73	14.79	15.07	0.75	3.37	3.09	0.19	100.00

Acetylcholinesterase Bimolecular Rate Constants ($M^{-1}min^{-1}$)

Global Human Orthogonal-PLS



Monte Carlo/Bootstrap Cross-Validation "Leave-random-number-out" Consensus QSAR Predictions



Global training R^2=0.91 using 74 significant and uncorrelated descriptors.

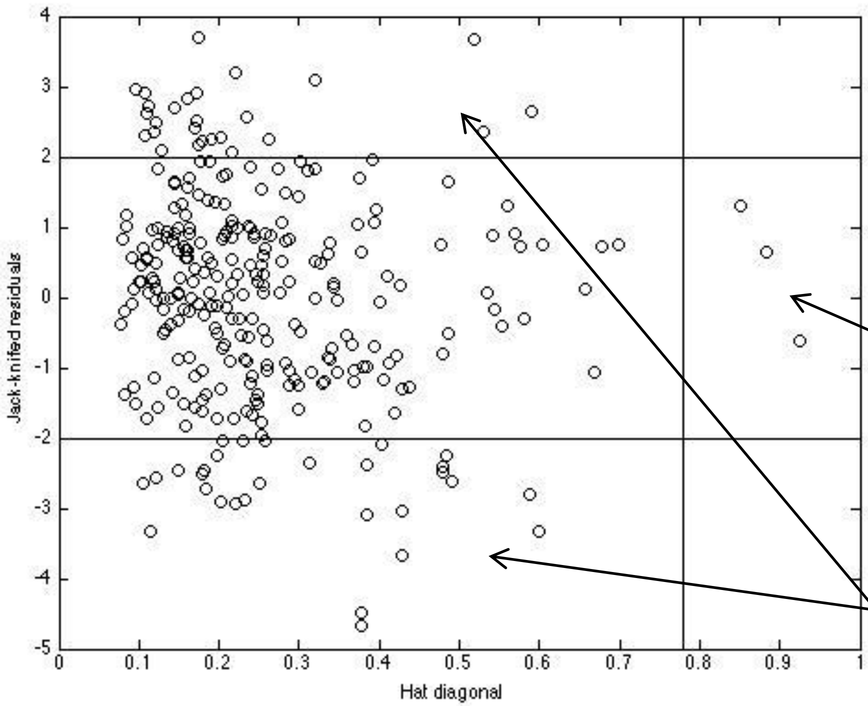
A mean training R^2 of 0.77±0.02 and an external test set Q^2 of 0.64±0.10 was achieved using the significant uncorrelated descriptors. Y-randomization Q^2=-0.23±0.18.

Domain of Applicability

- **A number of techniques exist to quantify the Domain of Applicability.**

QSAR model predictions are only valid within the applicability domain.

If your test compound falls within the DOA then you can expect a reliable prediction.



Compounds with high leverage can heavily influence a model. Predicted responses outside of the warning leverage may not be reliable.

Possible outliers

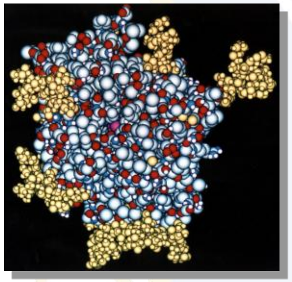
AChE Descriptor Significance

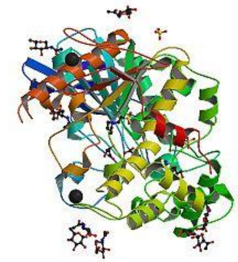
Descriptor Name	Normalized P value
Avg nucleoph. react. index for a C atom	1.000
HOMO energy	0.995
Min nucleoph. react. index for a O atom	0.991
Max nucleoph. react. index for a C atom	0.947
Max n-n repulsion for a C-H bond	0.889
(1/6)X GAMMA polarizability (DIP)	0.883
1X GAMMA polarizability (DIP)	0.883
Max e-n attraction for a C-H bond	0.831
HOMO - LUMO energy gap	-0.827
ESP-Max net atomic charge for a F atom	-0.827

Regression Techniques

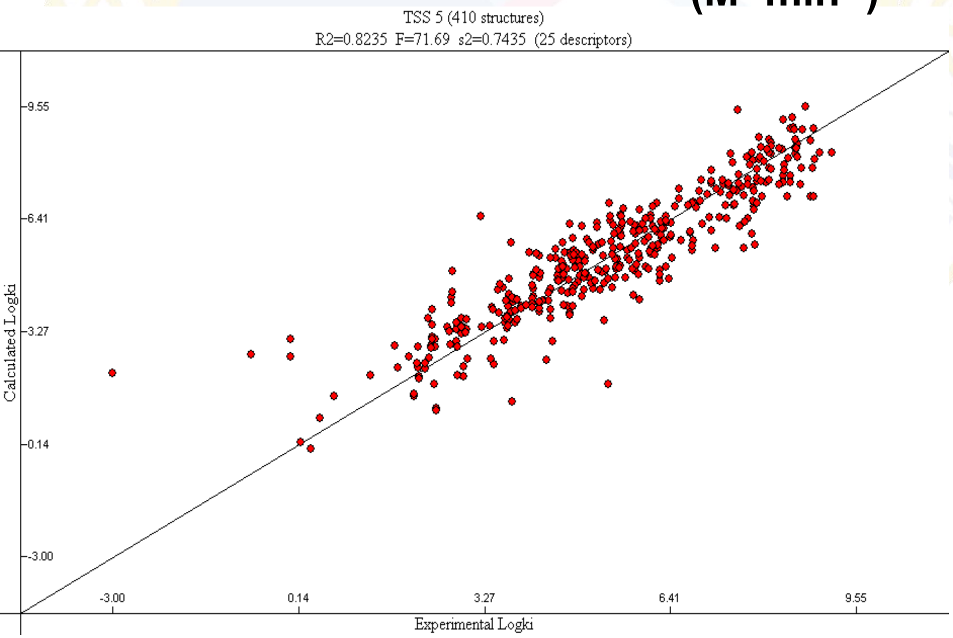
- **Heuristic (Built into CODESSA 2.51)**
  - Pre-selection of descriptors based upon a series of criteria cutoffs
    - Variation in descriptors
    - F-test
    - R^2
    - T-value
    - Inter-correlation
  - F-test measures significance of the whole model, t-test reflects significance of the parameter.

Butyrylcholinesterase "Serum Cholinesterase"

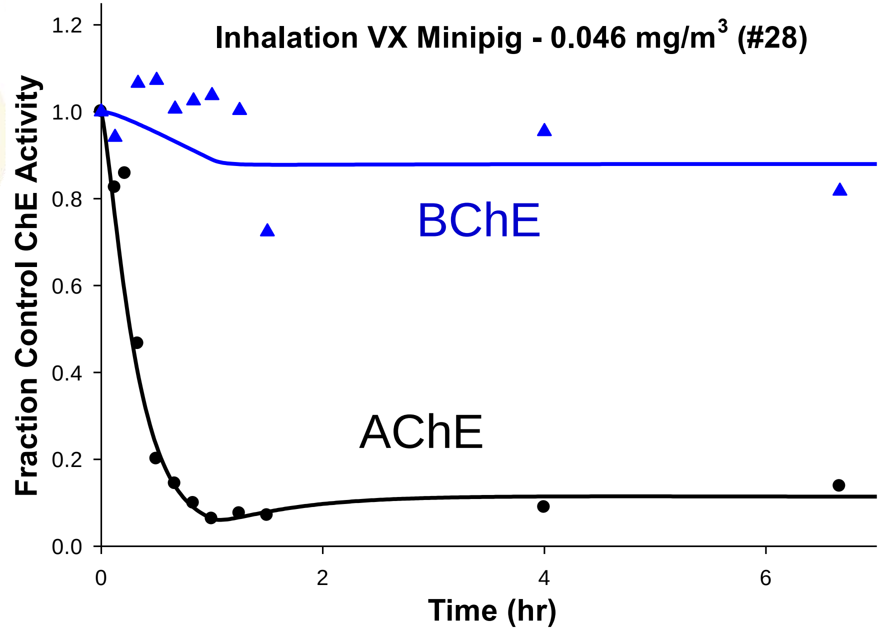




Bimolecular Rate ($M^{-1}min^{-1}$)

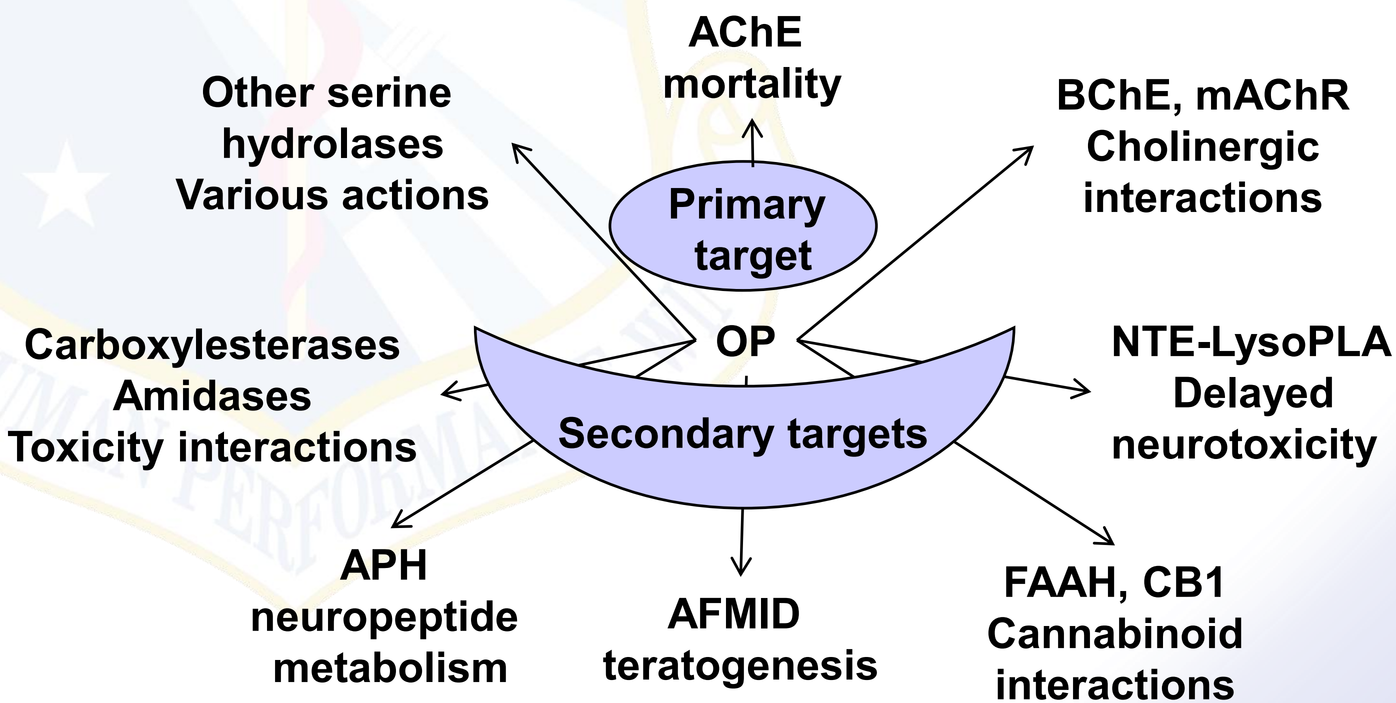


R^2=0.82, 25 descriptors, F=71.69, 410 compounds

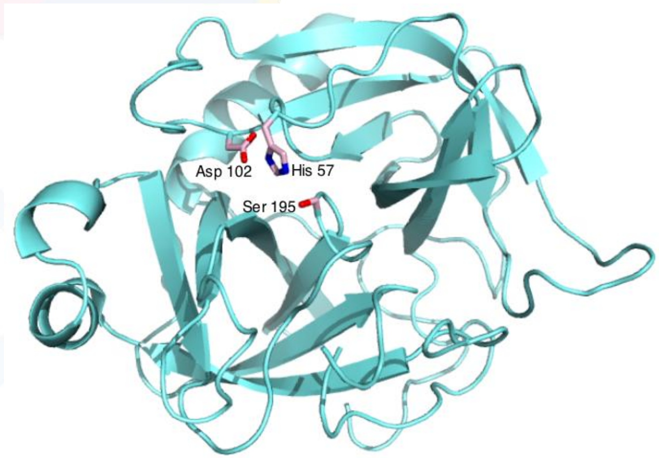


Data taken from literature.

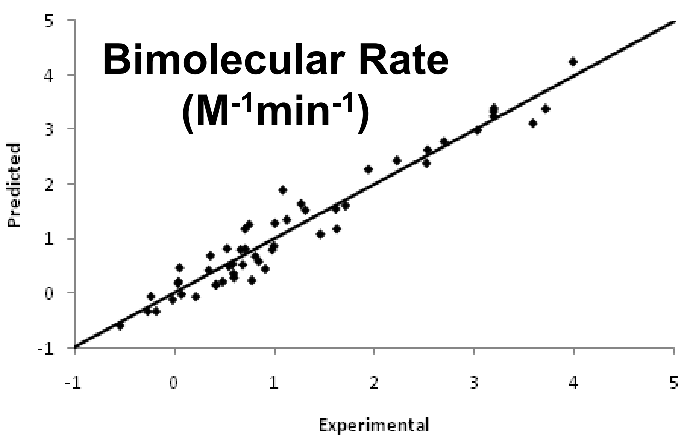
Noncholinergic Targets



Noncholinergic Targets
Digestive Proteases

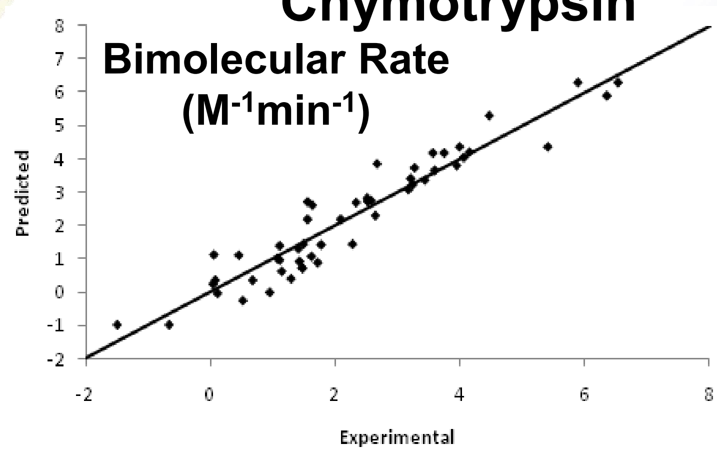


Trypsin



R^2=0.94, Q^2=0.90,
52 structures, 10 descriptors

Chymotrypsin



R^2=0.92, Q^2=0.87,
62 structures, 10 descriptors

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External Validation

Table 5. Trypsin results from the external validation using the ABC approach.

Training set	Number of compounds	R^2	Q^2	F	s^2	Test set	Number of compounds	R^2_{test}	$RMSE_{test}$
A + B	35	0.95	0.88	48.46	0.10	C	17	0.85	0.46
A + C	35	0.94	0.91	77.02	0.11	B	17	0.59	0.70
B + C	34	0.91	0.84	30.89	0.15	A	18	0.82	0.56
Average	34.67	0.93	0.88	52.12	0.12	Average	17.33	0.75	0.57

Table 6. α-Chymotrypsin results from the external validation using the ABC approach.

Training Set	Number of Compounds	R^2	Q^2	F	s^2	Test Set	Number of Compounds	R^2_{test}	$RMSE_{test}$
A + B	42	0.86	0.79	26.24	0.66	C	20	0.86	0.91
A + C	41	0.90	0.63	34.21	0.49	B	21	0.81	1.13
B + C	41	0.68	0.58	14.55	1.36	A	21	0.16	1.89
Average	41.33	0.81	0.67	25.00	0.84	Average	20.67	0.61	1.31

R^2=Coefficient of determination.

Q^2=Cross-validated LOO R^2.

F=Fisher F-test.

s^2=Mean squared error. $s^2 = \sum_{i=1}^{Ns}((Yic - Yio) * (Yic - Yio))/(Ns - Nd - 1)$ where Yic is the ith calculated/predicted property value, Yio is the ith observed/input property value, Ns is the number of training structures, Nd is the number of descriptors and the sum runs from 1 to Ns.

RMSE: Root mean standard error.

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Trypsin Descriptors

Descriptor Code	Descriptor Name	T-test (Global training set)	T-test (AB training set)	T-test (AC training set)	T-test (BC training set)
NA[a]	Error	4.40	3.23	-6.87	0.77
D_1	Number of F atoms	7.82	6.63	12.65	4.92
D_2	Kier shape index (order 2)	9.83	8.09	8.12	4.08
D_3	RNCG Relative negative charge (QMNEG/QTMINUS) [Zefirov's PC]	-0.84	0.10	8.33	1.80
D_4	Kier&Hall index (order 3)	-7.49	-5.06	-6.66	-3.80
D_5	Balaban index	-3.37	b	-2.65	-3.40
D_6	PPSA-3 Atomic charge weighted PPSA [Zefirov's PC]	-5.81	-4.75	b	-3.40
D_7	Number of O atoms	-5.24	-3.92	b	b
D_8	Relative number of H atoms	-5.11	-5.17	b	1.03
D_9	FPSA-1 Fractional PPSA (PPSA-1/TMSA) [Zefirov's PC]	3.66	3.10	b	b
D_{10}	Kier shape index (order 3)	2.00	2.08	-1.23	-0.49

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Conclusion

1. QSAR can be used to predict organophosphate oxon bimolecular rate constants for AChE, BChE, trypsin and chymotrypsin.

2. Approach can be applied to other PBPK/PD modeling parameters.

3. QSAR descriptors can provide a mechanistic description of the enzymatic reactions.

Steric hindrance, connectivity, lipophilicity, electrophilicity, electrostatics, hydrogen bonding, van der Waals

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